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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, 15/13, C07K 14/705, 14/73, 14/665, 14/745

A1

(11) International Publication Number: WO 99/45110

(43) International Publication Date: 10 September 1999 (10.09.99)

(21) International Application Number: PCT/AU99/00136

(22) International Filing Date: 5 March 1999 (05.03.99)

(30) Priority Data:
PP 2210 6 March 1998 (06.03.98) AU

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: V-LIKE DOMAIN BINDING MOLECULES

(57) Abstract

The present invention relates to novel binding moieties comprising at least one monomeric V-like domain (VLD) derived from a non-antibody ligand, the at least one monomeric V-like domain being characterised in that at least one CDR loop structure or part thereof is modified or replaced such that the solubility of the modified VLD is improved when compared with the unmodified VLD.

Claims:

1. A binding moiety comprising at least one monomeric V-like domain (VLD) derived from a non-antibody ligand, the at least one monomeric V-like domain being characterised in that at least one CDR loop structure or part thereof is modified or replaced such that the solubility of the modified VLD is improved when compared with the unmodified VLD.

2. A binding moiety according to claim 1 in which at least one CDR loop structure or part thereof is modified or replaced such that

(i) the size of the CDR loop structure is increased when compared with the corresponding CDR loop structure in the unmodified VLD; and/or

(ii) the modification or replacement results in the formation of a disulphide bond within or between one or more of the CDR loop structures.

3. A binding moiety comprising at least one monomeric V-like domain (VLD) derived from a non-antibody ligand, the at least one monomeric V-like domain being characterised in that at least one CDR loop structure or part thereof is modified or replaced such that

(i) the size of the CDR loop structure is altered when compared with the corresponding CDR loop structure in the unmodified VLD; and/or

(ii) the modification or replacement results in the formation of a disulphide bond within or between one or more of the CDR loop structures.

4. A binding moiety according to claim 3 in which the size of the CDR loop structure is increased by at least two amino acid residues.

5. A binding moiety according to claim 3 in which the size of the CDR loop structure is increased by at least six amino acid residues.

6. A binding moiety according to claim 3 in which the size of the CDR loop structure is increased by at least nine amino acid residues.

7. A binding moiety according to any one of claims 1 to 6 in which the binding affinity of the modified VLD is altered when compared with the unmodified VLD.

8. A binding moiety according to claim 7 in which the affinity of the modified VLD to at least one natural ligand of the unmodified VLD is reduced.

9. A binding moiety according to any one of claims 1 to 8 in which the binding specificity of the modified VLD is different to that of the unmodified VLD.

10. A binding moiety according to any one of claims 1 to 9 in which the non-antibody ligand is a T-cell surface protein.

11. A binding moiety according to claim 10 in which the non-antibody ligand is CTLA-4, CD28 or ICOS.

12. A binding moiety according to claim 11 in which the non-antibody ligand is CTLA-4.

13. A binding moiety according to any one of claims 1 to 12 in which one or more of the CDR loop structures is replaced with a binding determinant derived from a non-antibody polypeptide.

14. A binding moiety according to claim 13 in which the binding determinant is derived from somatostatin or haemagglutinin.

15. A binding moiety according to any one of claims 1 to 12 in which one or more of the CDR loop structures is replaced with one or more CDR loop structures derived from an antibody or antibodies.

16. A binding moiety according to claim 15 in which the antibody or antibodies are derived from a rat, mouse, human, camel, llama or shark.

17. A binding moiety according to claim 15 or claim 16 in which the antibody or antibodies are selected from the camel antibody cAB-Lys3 and the human anti-melanoma antibody V86.

18. A binding moiety according to any one of claims 1 to 17 linked to a diagnostic reagent.

19. A binding moiety according to claim 18 in which the diagnostic reagent is selected from the group consisting of streptavidin, biotin , a radioisotope, dye marker or other imaging reagent.

20. A multivalent reagent comprising two or more binding moieties as claimed in any one of claims 1 to 19.

21. A binding moiety or multivalent reagent according to any one of claims 1 to 20 immobilised on a solid support or coupled to a biosensor surface.

22. A polynucleotide encoding a binding moiety or multivalent reagent as claimed in any one of claims 1 to 20.

23. A vector comprising a polynucleotide according to claim 22.

24. A host cell transformed with a vector as claimed in claim 23.

25. A host cell according to claim 24 in which the cell is a bacterial cell.

26. A method of producing a binding moiety which comprises culturing a host cell as claimed in claim 24 or claim 25 under conditions enabling expression of the binding moiety and optionally recovering the binding moiety.

27. A method according to claim 26 in which the binding moiety is unglycosylated.

28. A pharmaceutical composition comprising a binding moiety as claimed in any one of claims 1 to 20 and a pharmaceutically acceptable carrier or diluent.

29. A method of treating a pathological condition in a subject, which method comprises administering to the subject a binding moiety as claimed in any one of claims 1 to 20.

30. A method of selecting a binding moiety with an affinity for a target molecule which comprises screening a library of polynucleotides for expression of a binding moiety with an affinity for the target molecule, the polynucleotides encoding VLDs derived from one or more non-antibody ligands, wherein the polynucleotides have been subjected to mutagenesis which results in a modification or replacement in at least one CDR loop structure in at least one VLD and wherein the solubility of the isolated modified VLD is improved when compared with the isolated unmodified VLD.

31. A method according to claim 30 in which the screening process involves displaying the modified V-like domains as gene III protein fusions on the surface of bacteriophage particles.

32. A method according to claim 30 in which the screening process involves displaying the modified V-like domains in a ribosomal display selection system.

33. A binding moiety according to any one of claims 1 to 20 produced by a method according to any one of claims 30 to 32.